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Fuzzy and fast nuclear transport

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Exchange of macromolecules between the cytoplasm and the nucleus of all eukaryotic cells is controlled by nuclear pore complexes, which form a selective permeability barrier. The requirement for rapid but selective transport leads to a "transport paradox." A new experimental study now provides a thermodynamic explanation.

Vital functions such as gene expression, cell growth, and cell division critically depend on the continuous passage of molecules from the nucleus to the cytoplasm and vice versa. Nuclear pore complexes (NPCs)[2] mediate this molecular exchange and thereby play a pivotal role in the organization of cellular molecular traffic. Structurally, the NPC is an amazing macromolecular assembly of 60–120 MDa, consisting of at least 30 different nucleoporin proteins (Nups) that unite into an hourglass-shaped architecture (see, for example, Ref. 1, for a recent review on NPC structure and function). Central to the nucleocytoplasmic transport are the FG Nups, intrinsically disordered polypeptides (IDPs) that together contribute about 3000–4000 phenylalanine–glycine (FG) repeats to the central aperture and form a permeability barrier. This barrier does not substantially impact traffic of smaller molecules, which may transit the pore relatively unimpeded by passive diffusion. However, larger cargo requires assistance to pass the FG Nups in the form of soluble transport factors (TFs) that transiently bind to the FG Nups and carry them through the NPC in an energy-coupled manner. Despite its importance, this transport mechanism is poorly understood both structurally and mechanistically. In particular, FG Nups must achieve selective transport of cargo, while at the same time facilitating rapid transit to support cellular functions. This poses a potential problem, as protein–protein interactions are typically either weak and nonspecific or strong and selective. How then do FG Nups break this "transport paradox"?

In this exciting Accelerated Communication, Cowburn and co-workers (2) provide a possible explanation by carefully examining the energetics of multivalent interactions using a combination of calorimetry and NMR spectroscopy. The team characterized in exquisite detail the interactions between yeast nuclear transport factor 2 (NTF2) and constructs with variable numbers of FSFG motifs. First, the authors show that individual FSFG-mediated interactions are of low affinity and that multiplexing the number of interaction sites does not lead to a synergistic increase in affinity. This is not necessarily expected, as multivalent interactions may well lead to such "avidity." The apparent lack of cooperativity of binding between NTF2 and multiple FSFG motifs suggests that the system may be undergoing rapid unbinding and rebinding in multiple alternative ways, resulting in short lifetimes of individual low-affinity complexes. At the same time, the FG motifs are tethered together, which results in an increase in the local concentration of interaction sites, resulting in a selective clasp on the TF.

The authors substantiated this result by inspecting the effect of the distance between motifs on binding between the transport factor and the constructs. As expected, closely spaced motifs had greater overall affinity than segments that were farther apart. Finally, to understand what prevented strong avidity, the thermodynamics of the interactions is elucidated by calorimetry. Surprisingly, the hydrophobic FG–TF associations were driven by enthalpy, with each new motif increasing the magnitude of ΔH. Nearly quantitative enthalpy–entropy compensation was observed, explaining why the multivalent complexes showed affinities that were not more than additive. The "transport paradox" is thus resolved by Hayama *et al.* (2) in the following way: A high local concentration of FG repeats with short interaction half-lives results in a high frequency of contacts, thereby increasing the effective lifetime of two interaction partners of appropriate overall complementarity. The entropy loss associated with the binding of additional FG elements helps to prevent overly strong association and thereby provides a principle for highly dynamic interactions to maintain transport speed through the nuclear pore complex. The term "kiss-and-run" has previously been used in this context (1), and the data presented in Ref. 2 attest to the high promiscuity on the part of the IDP, where multiple similar interaction motifs may continuously exchange to pass on the partner (Fig. 1).

The present paper is of significant impact for our understanding of transport through the NPC, but the conclusions from this paper reach much wider. Specifically, the insights provided here more generally influence our comprehension of interactions involving intrinsically disordered proteins. IDPs constitute both a large and important part of eukaryotic biology and are also directly implicated in many human disorders. As their relative abundance in the human proteome completely

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[2] The abbreviations used are: NPC, nuclear pore complex; IDP, intrinsically disordered protein; TF, transport factor; FG Nup, Phe–Gly nucleoporin.

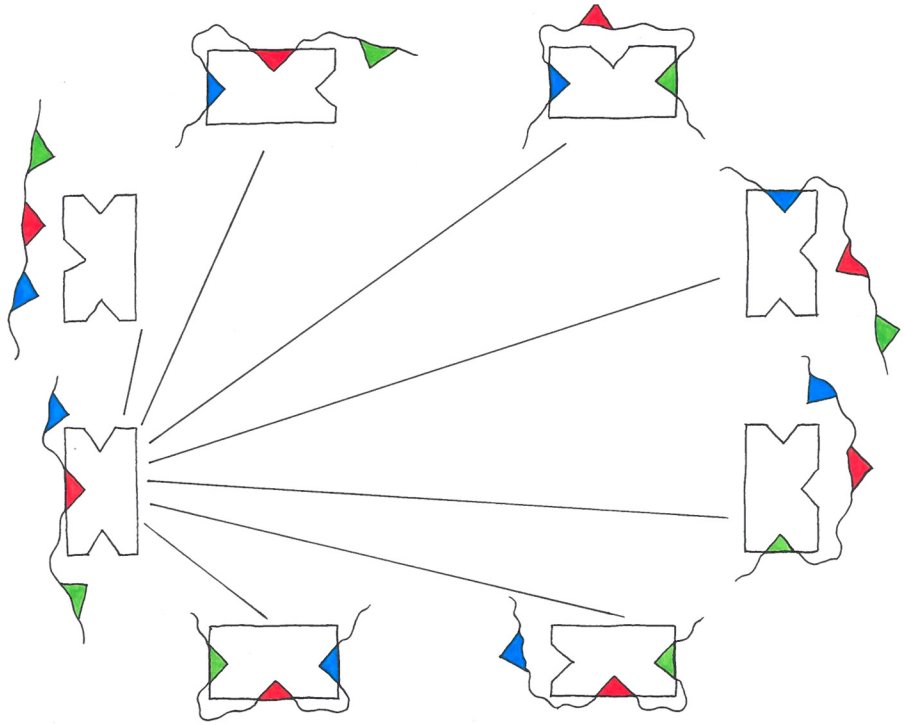

Figure 1. Illustration of the possible multivalent interactions made by IDPs like the FG sequences in FG Nups with binding partners like the nuclear pore transport factors. Binding partners (*white rectangle*) possess several (degenerate) interaction motifs, allowing multiple weak interactions with unstructured motifs (*colored triangles*). Enthalpy–entropy compensation prevents the strong engagement of many motifs simultaneously, while concurrently securing rapid dissociation. The close, tethered spatial organization of multiple motifs increases local concentration, and selective recognition results from an increase in the overall time that two particular partners remain united.

mismatches our current perception and appreciation of them, this "dark side of the proteome" (3) constitutes a rapidly expanding area of research. IDPs are disordered and flexible and therefore elusive to X-ray crystallography. Instead, IDPs are characterized by ensembles, mainly derived from NMR restraints. However, as the current work elegantly shows, not only *structural*, but also *thermodynamic* insights are pivotal in helping us move forward. Multivalency concepts of molecular engagement and interaction have been much less engrained in our thinking than the powerful lock-and-key concept of Emil Fischer (4). As more data are collected, the immense limitations of static views without a thermodynamic foundation become undeniably apparent (5). To understand intrinsic disorder, we must embrace concepts like polyvalent interactions (6), along with thermodynamic experiments and models (7). The work of Hayama *et al.* (2) on FSFG–NTF2 interactions shows how critical thinking and experiments pave the way to a comprehensive understanding of key biological functions.

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